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Seroepidemiological Study of Genogroup I and II Calicivirus Infections in South and Southern Africa

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Diarrhoea is associated with the daily death of between 180 and 200 children under the age of 5 years in South Africa. Until recently, many cases and outbreaks of diarrhoea were not associated with a known aetiologic agent. Previous studies using baculovirus-expressed Norwalk virus (NV) and Mexico virus (MxV) capsid antigens have shown that human calicivirus infection is common in South Africa. In this study, our surveillance was extended to different populations, as well as to four other southern African countries: Namibia, Angola, Zimbabwe, and Mozambique. More than 1,700 specimens, some involved in previous cohort studies of infectious diseases, were enrolled in the surveillance. The overall seroprevalence of antibody against NV was >90% for all cohorts except for Mozambican refugees that had 83.8% sero-positivity. The MxV antibody prevalence was higher than NV, with >95% positivity for all cohorts, except for one in Namibia that had 81% exposure. This study is one of only a few reporting on the concurrent incidence of NV and MxV infections in a cohort study, and has determined that small round structured viruses are prevalent in the local populations of South and Southern Africa. These agents may account for a number of previously unknown or unidentified causes of diarrhoeal illness, in both adults and children, in southern Africa. ***J. Med. Virol. 59:227–231, 1999.***

KEY WORDS: SRSVs; Norwalk virus; Mexico virus; seroprevalence

INTRODUCTION

Caliciviruses are enteric pathogens and have been associated with various disease syndromes and subclinical infections in a number of mammalian and avian species. Caliciviruses infecting humans include two forms based on their morphology: the typical or "classical" caliciviruses with distinctive morphology, and atypical or small round structured viruses (SRSVs), which are less clearly defined by electron microscopy. Molecular characterisation by cloning and sequencing of the prototype Norwalk virus (NV) and many related strains has led to the classification of SRSV in the *Caliciviridae* family [Jiang et al., 1993, 1995a; Lambden et al., 1993; Lew et al., 1994a, 1994b; Cubitt et al., 1995].

Based on the sequence similarities in the RNA-dependent RNA polymerase and the capsid regions of the viral genome, the SRSVs have been divided further into two distinct genogroups: the NV and the Snow Mountain virus (SMV) genogroups, or genogroups I and II, respectively. The NV genogroup includes the prototype NV, Southampton virus, and the Desert Shield virus [Jiang et al., 1993; Lambden et al., 1993; Lew et al., 1994a; Wang et al., 1994]. The SMV genogroup includes the human prototype SMV, Hawaii, Toronto, Bristol, Lordsdale, and Mexico viruses [Madore et al., 1986; Green et al., 1994; Lew et al., 1994b; Dingle et al., 1995; Jiang et al., 1995b]. This genetic classification is known to correlate with the antigenic types of SRSVs. Based on the results by recombinant enzyme immunoassays, NV and SMV genogroups are antigenically distinct with a low level of shared antigenic epitopes [Jiang et al., 1996].

Diarrhoeal disease is a major cause of morbidity and mortality worldwide [Bern and Glass, 1994]. In South Africa, between 180 and 200 children (<5 years of age) die daily as a result of diarrhoea [Wittenberg, 1997]. Until recently, many cases and outbreaks of diarrhoea, in which viral pathogens are believed to play an important role, have been associated with an unknown aetiologic agent [Steele et al., 1988]. The importance of

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TABLE I. Demographic Details of the Population Cohorts Tested for Antibodies to Norwalk Virus and Mexico Virus by Recombinant ELISA

Region	Description	No.	Age (years)	M:F	Original source	Percent positive anti-NV	Percent positive anti-MxV
Gauteng: urban	Family cohort	488	0–87	215:273	Manyike et al., 1990	**98**	**99**
Gauteng: rural	ANC, FPC, mothers	360	15–51	Female	Steele et al., 1995	**96**	**96**
	at delivery	326	17–49	Female		**95**	**96**
Gauteng: babies	Cord bloods	92	At birth	Not given		**90**	**91**
Vendaland	School children	360	6–21	169:192	Not described previously	**98**	**99**
Schmidtsdrift	San family cohort	352	1–83	143:207	Gaobepe et al., 1995	**94**	94.5
Ovamboland	Family cohort	187	0–88	85:102	Botha et al., 1984	98.4	81
East Caprivi	Family cohort	393	4–65	205:187	Joubert et al., 1991	96.5	91
Bushmanland	San family cohort	294	3–65	172:119	Steele et al., 1994	91.8	nd[a]
Mozambique	Family refugees	365	3–79	141:223	Bos et al., 1995	83.8	nd[a]
Mucossa, Angola	Adult volunteers	201	Adult	120:79	Bos and Steele, 1996	99	93
Zimbabwe	Clinic attendees	273	Adult	Unknown	Not described previously	98.5	nd[b]

ELISA, enzyme-linked immunosorbent assay; ANC, antenatal clinic; FPC, family planning clinic; nd, not determined.
[a]These samples could not be analysed due to insufficient volumes, deterioration of the samples due to long-term storage and repeated freeze-thawing.
[b]These samples were not available for testing.
The values in boldface represent data presented in part by Steele et al. at the First International Symposium on Caliciviridae, Reading, UK, 1997.

rotavirus and adenovirus as causes of nonbacterial acute gastroenteritis in children and adults is well recognised. However, due to a lack of suitable methods and reagents for diagnosis of human caliciviruses, the importance of the group of SRSVs remains to be determined in many parts of the world, including Africa.

The successful expression of recombinant virus-like particles of NV, Mexico virus (MxV), and other strains of SRSVs in a baculovirus expression system has enabled the development of sensitive and specific immunological assays for the diagnosis of human caliciviruses [Parker et al., 1993; Jiang et al., 1995a]. These assays have led to the widespread investigation of the seroepidemiology of SRSVs in many countries, including the UK, Japan, Papua New Guinea, Mexico, Finland, Saudi Arabia, and Kuwait [Gray et al., 1993; Lew et al., 1994c; Numata et al., 1994; Parker et al., 1994, 1995; Jiang et al., 1995a; Dimitrov et al., 1997]. These studies have shown that infection with SRSVs is worldwide and that they are a major cause of acute gastroenteritis and vomiting disease in children.

A higher antibody prevalence to SRSVs has been reported in developing than developed countries [Numata et al., 1994; Parker et al., 1994; Dimitrov et al., 1997; Smit et al., 1997]. Furthermore, the age of acquisition of antibodies to SRSVs differs among different countries. For example, NV infection occurs mostly in teenagers and adults in Japan [Numata et al., 1994]; in children between the ages of 2 and 10 years in the UK [Gray et al., 1993; Parker et al., 1994]; and in younger children in Mexico, Kuwait, and South Africa [Jiang et al., 1995a; Dimitrov et al., 1997; Smit et al., 1997).

Recently, the seroprevalence of antibodies against NV and MxV was reported in several cohorts in South Africa [Smit et al., 1997; Steele et al., 1997]. A high prevalence of serum antibodies against NV and MxV was observed in these cohorts, as reported for other developing communities and countries. Furthermore, most children had developed antibodies to both NV and MxV by the age of 48 months. However, in another study conducted in South Africa, NV antibodies were present in only 56% of persons of European origin, and in 53% of the persons of ethnic origin [Taylor et al., 1996]. The reasons for these differences are not known at present. A study by Wolfaardt et al. [1995] determined, by reverse-transcriptase polymerase chain reaction (RT-PCR) and sequencing, that the dominant strain circulating in South Africa was strongly related to MxV.

In this study, we extended our surveillance to different populations, as well as to four other southern African countries. A similar high prevalence of serum antibodies against two strains of SRSVs was observed in all locations studied.

MATERIALS AND METHODS

Serum Samples

Serum samples were available from a number of separate population cohorts in South and southern Africa as detailed in Table I. Several of the cohorts were available from previous hepatitis B vaccine trials conducted in our laboratory. These included the cohorts of school children from Vendaland, and the healthy family-based cohorts from Gauteng, Schmidtsdrift, Ovamboland, and East Caprivi. In addition, sera collected from women attending the antenatal and family planning clinics at Ga-Rankuwa Hospital were available from the diagnostic laboratory for testing. The sera from Angola and Zimbabwe were collected from adult volunteers.

Recombinant NV and MxV Antibody EIAs

All serum samples were tested for the presence of antibodies to NV and MxV using a direct sandwich enzyme immunoassays (EIAs) as described previously [Parker et al., 1993, 1994; Jiang et al., 1995a, 1995c].

Briefly, test sera diluted at 1:100 and three negative controls were added to wells of a 96-well polyvinyl chlo-

TABLE II. Prevalence and Age Distribution of IgG Antibodies to Norwalk Virus and Mexico Virus in Different Southern African Population Groups

Age range (years)	Number tested	Norwalk virus			Mexico virus		
		No. positive	Percent	95% CI	No. positive	Percent	95% CI
1–2	4	4	100	100–100	3	75.0	32.5–1.17
3–4	27	24	88.9	77.03–100.74	24	88.9	77.03–100.74
5	41	37	90.2	81.16–99.33	38	92.7	84.71–100.65
6	98	92	93.9	89.13–98.62	95	96.9	93.53–100.35
7	136	134	98.5	96.51–100.55	132	97.1	94.22–99.99
8	127	118	92.9	88.45–97.38	123	96.8	93.81–99.89
9	129	122	94.6	90.66–98.48	125	96.9	93.91–99.89
10–14	572	540	94.4	92.52–96.29	557	97.4	96.07–98.69
15–19	348	337	96.8	95.00–98.68	339	97.4	95.75–99.08
20–29	294	278	94.6	92.00–97.15	281	95.6	93.23–97.93
30–39	220	203	92.3	88.74–95.80	213	96.8	94.50–99.14
40–49	133	121	90.9	86.11–95.85	127	95.5	91.96–99.02
50–59	101	95	94.1	89.45–98.67	94	93.1	88.12–98.02
60–69	87	82	94.3	89.36–99.14	85	97.7	94.55–100.85
>70	41	38	92.7	84.71–100.65	40	97.6	92.84–102.28
Total	2,358	2,225	94.4	93.43–95.29	2,276	96.5	95.78–97.26

CI, confidence interval.

ride flat-bottom microtitre plate (Immulon-2, Dynatech) coated with either baculovirus-expressed recombinant NV or MxV capsid protein. Three negative samples were added per plate. An alkaline-phosphatase-conjugated goat anti-human immunoglobulin G, M, and A (Zymed) was used as detector antibody and 3,3′,5,5′-tetramethylbenzidine (TMB enzymatic kit, Roche) was used as the substrate. After development of the enzymatic reaction, the reaction was stopped with sulphuric acid and the absorbance read spectrophotometrically at 450 nm. Test reactions with absorbance values greater than twice the mean of the negative control sera and ≥ 0.100 were considered positive.

RESULTS AND DISCUSSION

The results obtained for the seroprevalence study of antibodies to NV and MxV in the various population cohorts of South and southern Africa are shown in Table I. In all but two cohorts, the prevalence of antibodies to these two viruses exceeded 90%. Specifically, in all cohorts, except the Mozambican refugees, antibodies to NV were detected in more than 90% of the serum samples. In the cohort of Mozambican refugees, only 83.8% had anti-NV antibodies. Unfortunately, there were insufficient sera available to screen this group for antibodies to MxV due to deterioration of the samples and in some cases insufficient material remaining. Nevertheless, it may be suggested that the lower levels of antibody in this group was a long-term effect of malnutrition and immune depression from their flight from Mozambique.

Similarly, in all cohorts, except the Ovamboland family cohort, antibody to MxV was detected in more than 94% of the serum samples. Only 81% of the samples from the Ovamboland cohort had anti-MxV antibodies. This group lives in a fairly remote area of Namibia and a possible explanation is that there was less exposure to MxV in this region, although why this difference in exposure should be so is unknown.

This study is one of only a few reporting on the concurrent incidence of NV and MxV infections in a cohort study, and has determined that SRSVs are prevalent in the local populations of South and southern Africa. Anti-NV and anti-MxV antibodies were observed to be present at very high levels in each of the population cohorts examined. The levels of exposure to these viruses were similar to that reported for Kuwaitis (98% and 96%, respectively) and for foreign workers in Kuwait (98% and 95%, respectively) [Dimitrov et al., 1997]. Antibodies against NV, which has been examined more extensively, were also high in Panama [98%, Ryder et al., 1985], Indonesia, Papua New Guinea [90% and 100%, Numata et al., 1994], and Australian aborigines [94%, Parker et al., 1994].

High levels of antibody were detectable across the full age range in each cohort, including the 3- and 4-year-old children in the East Caprivi and Bushmanland cohorts (Table II). This finding suggests that the children were already infected at an earlier age, as we reported previously for the Ga-Rankuwa, Gauteng cohort [Smit et al., 1997; Steele et al., 1997]. This pattern is similar to that observed in developing communities and countries, such as Bangladesh, Mexico, and Kuwait, and among the Australian Aborigines, where children generally acquire the infection by 24 months of age. In developed countries such as Japan, the United Kingdom, and the USA, antibodies to NV seem to be acquired later by school-aged children (5 or 6 years of age).

The prevalence of antibody to NV in these African populations, both rural and urban, is amongst the highest recorded. The high levels of antibody to NV are different from that reported for this virus in some developed countries such as the USA [Greenberg et al., 1979], the UK [Parker et al., 1993], Finland [Lew et al., 1994c], and Singapore and Japan [Numata et al., 1994]. Similar low levels of anti-NV was also previously reported in South Africa in both European

(56.4%) and African (53.9%) groups in South Africa [Taylor et al., 1996], although a large proportion of these cohorts were young children. However, the results found in this study, when coupled with other recent reports from developing countries, such as Brazil, Mexico, and Kuwait [Gabbay et al., 1994; Jiang et al., 1995c; Dimitrov et al., 1997], indicate that NV is circulating at high levels in these communities.

Several viral agents cause diarrhoeal illness in humans. In South Africa, rotaviruses and adenoviruses have been well documented to be associated with acute infantile gastroenteritis [Kidd et al., 1986; Steele et al., 1986, 1988]. Human astrovirus has also been reported to be common in young children with diarrhoea [Steele et al., 1998] and to be associated with outbreaks in Day Care Centres [Marx et al., 1998]. However, relatively little is known of the role of the human caliciviruses in acute nonbacterial infantile gastroenteritis. Two outbreaks of diarrhoea have been reported in adults indicating the circulation of viral agents related to both NV and MxV in this region [Taylor et al., 1993]. Furthermore, two separate studies have determined that MxV-like strains are associated with disease in young children in South Africa, although apparently at low levels [Wolfaardt et al., 1995; Steele et al., 1997].

Nonetheless, the incidence of antibodies to both NV and MxV in this study indicates that both genogroup I (NV-like) and genogroup II (Snow Mountain agent-like) viruses are circulating, probably simultaneously, in South and southern Africa. This study has provided more extensive epidemiological data on the prevalence of SRSVs and evidence that these agents may account for a number of previously unknown or unidentified causes of diarrhoeal illness, in both adults and children.

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